Water Standards and Studies Report #87-3

Effects of Aerial Spraying on Forestry Herbicides on Aquatic Ecosystems

Part III. Bioassay of the effect of glyphosate on carbon fixation by intact periphyton communities

By L.G. Goldsborough and D.J. Brown

Manitoba
Environment and
Workplace Safety
and Health



Effects of Aerial Spraying of Forestry Herbicides on Aquatic Ecosystems

Part III. Bioassay of the effect of glyphosate on carbon fixation by intact periphyton communities

L.G.Goldsborough & D.J.Brown
Water Standards and Studies Section
Environmental Management Services Branch
Environmental Management Division

Department of Environment and Workplace Safety and Health
Water Standards and Studies, Report #87-3

Goldsborough,L.G.[1] and D.J. Brown[2]. 1987. Effects of aerial spraying of forestry herbicides on aquatic ecosystems. Part III. Bioassay of the effect of glyphosate on carbon fixation by intact periphyton communities. 27pp.

Abstract

As part of a three-year study examining potential effects of herbicide use in forest management on adjacent aquatic ecosystems, artificial substrata (acrylic rods) were positioned in shallow water within six experimental study ponds near Pine Falls (96° 10'W, 50° 37'N) during late summer. The substrata were permitted to colonize with periphytic algae for a period of 41 to 50 days, after which samples were collected and exposed in the laboratory to concentrations of glyphosate (added as the commercial formulation Roundup) ranging from 0 to 1800 mg/L. Carbon fixation rates during the first four hours after herbicide treatment were measured, and the change in rate relative to untreated controls was used to assess the short-term effects of glyphosate on algal photosynthesis.

Effects of glyphosate treatment on carbon fixation were not detected in samples from any pond at concentrations less than about 0.89 mg/L. Carbon fixation occurred at the highest tested concentration. Estimated EC_{50} values for Pine, Hike and Birch Ponds ranged from 35.4 to 69.7 mg/L glyphosate. Based on the estimated glyphosate concentration which could result from a hypothetical application of 2.5 L/ha Roundup to the study ponds (0.06-0.10 mg/L), no immediate effects of herbicide treatment on periphytic algal photosynthesis are predicted.

[1] Present Address: Department of Botany
University of Alberta
Edmonton, Alberta
T6G 2E9

[2] Correspondence Address: Water Standards and Studies Section
Manitoba Environment and Workplace Safety and Health
Box 7, Building 2, 139 Tuxedo Avenue
Winnipeg, Manitoba
R3N 0H6

Table of Contents

	Page
Abstract	i
Table of Contents	ii
List of Figures	iii
List of Tables	iv
List of Appendices	iv
Acknowledgements	v
1.0 Introduction	1
2.0 Materials & Methods	4
3.0 Results	8
4.0 Discussion	13
5.0 Literature Cited	22

List of Figures

Page

Figure 1: Locations (■) of artificial substrata sampling stations in the six study ponds, Pine Falls, 1985. Two sets of substrata were positioned at the Birch Pond station. Ponds are not shown in their correct geographic locations 6

Figure 2: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Birch Pond on 8 October, 1985 10

Figure 3: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Spruce Pond on 11 October, 1985 11

Figure 4: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Raspberry Pond on 11 October, 1985 12

Figure 5: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Pine Pond on 14 October, 1985 14

Figure 6: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Golden Pond on 14 October, 1985 15

Figure 7: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Hike Pond on 17 October, 1985 16

Figure 8: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Birch Pond on 17 October, 1985 17

List of Tables

Page

Table 1: Chemical and physical properties of glyphosate and the commercial formulation (Roundup) 2

Table 2: Surface water chemistry at the time of periphyton sample collection from the six study ponds (8-17 Oct. 1985) 5

Table 3: Estimated biomass of periphytic algae on artificial substrata (17.5-30 cm above base) collected from study ponds, based on total chlorophyll *a* concentration and carbon fixation rate (4-hour incubation) 9

Table 4: Hypothetical glyphosate concentration (mg/L) in water of the study ponds after a 2.5 L/ha Roundup (0.89 kg a.i./ ha) application 21

List of Appendices

Page

Appendix A: Carbon fixation rates (ug/cm²/h) of periphyton samples (3 replicates) collected from Birch Pond (preliminary experiment - 8 October, 1985) in response to glyphosate (Roundup) additions 26

Appendix B: Carbon fixation rates (ug/cm²/h) of periphyton samples (3 replicates) collected from Spruce and Raspberry Ponds (11 October, 1985) in response to glyphosate (Round up) additions 26

Appendix C: Carbon fixation rates (ug/cm²/h) of periphyton samples (3 replicates) collected from Pine and Golden Ponds (14 October, 1985) in response to glyphosate (Roundup) additions 27

Appendix D: Carbon fixation rates (ug/cm²/h) of periphyton samples (3 replicates) collected from Hike and Birch Ponds (17 October, 1985) in response to glyphosate (Roundup) additions 27

Acknowledgements

Financial support for this project was made available through the Canada-Manitoba Forest Renewal Agreement.

We gratefully acknowledge the field assistance of Al Beck, Colin Hughes and Dwight Williamson. Abitibi-Price Inc. (Pine Falls) kindly provided living facilities and assistance in locating the study ponds. Dr.Gordon Robinson (Dept of Botany, University of Manitoba) generously provided laboratory facilities and equipment.

1.0 Introduction

Glyphosate is a non-selective, broad spectrum, post-emergent herbicide recognized for use in the control of grasses, sedges and broad-leaved weeds (WSSA 1983). It has received widespread use in agricultural and silvicultural practise in Canada, the United States and elsewhere. It was originally synthesized and is marketed as a commercial formulation by Monsanto Company of St.Louis, Missouri under the brand name Roundup (WSSA 1983).

Glyphosate is a weak acid, is water soluble and is readily bound to soil due to the presence of a phosphorus moiety in the herbicide molecule (Sprankle et al. 1975a; Hance 1976). A summary of its primary chemical properties is given in Table 1. A comprehensive literature review of the toxicological effects of glyphosate may be found in Grossbard & Atkinson (1985). The low molecular weight and moderate water solubility appear to facilitate rapid absorption and both basipetal and acropetal translocation in vascular plants (Hoagland & Duke 1982; Sprankle et al. 1975c). Although the primary mode of action of glyphosate has not yet been definitively established, it is believed to interfere with aromatic amino acid biosynthesis in bacteria, algae and higher plants through effects on the enzymes chlorismate mutase and prephenate dehydratase (Jaworski 1972) or 5-enolpyruvyl shikimate-3-phosphate synthase (Steinrucken & Amrhein 1980). Depletion of cellular aromatic amino acids reduces or stops protein synthesis, leading to cessation of growth (Hoagland & Duke 1982). Glyphosate-induced effects may often be counteracted by additions of phenylalanine and/or tyrosine (Chan & Leung 1986). Effects of glyphosate on photosynthesis have also been reported. Van Rensen (1974) concluded that the herbicide directly interferes with electron transport in photosystem II, while Richard et al. (1979) suggested this effect could be an artifact of changes in the pH of culture media due to glyphosate additions. The latter authors reported no effect of glyphosate on photosynthetic response of isolated Pisum sativum chloroplasts in pH-adjusted medium.

Concern has been raised of the potential adverse environmental effects on offsite movement (terrestrial runoff, baseflow or spray drift from aerial applications) of glyphosate, particularly where treatment areas border bodies of water. Studies of the persistence and fate of glyphosate in freshwater have focussed on lotic systems (Bowmer et al. 1986; Newton et al. 1984; Comes et al. 1976), where decreases in herbicide concentration with time are expected. Immediately following treatment of an Oregon forest stream with 9.3 L/ha Roundup (ca. 3.3 kg glyphosate/ha), Newton et al. (1984) reported a maximum glyphosate concentrations of about 0.27 mg/L. Wan (1986) reported a concentration of 0.023 mg/L glyphosate in a British Columbia forest stream 2-3 hours following application of 3 kg glyphosate/hectare. Following a rainstorm, a maximum concentration of 0.100 mg/L was detected in stream flow. Where a buffer zone adjacent to stream banks (10-100 m width) was established, glyphosate was lower than 0.025 mg/L at all times.

Table 1: Chemical and physical properties of glyphosate and the commercial formulation (Roundup)

compound name:	glyphosate
chemical name:	N-(phosphonomethyl)glycine[1]
molecular weight:	169.1[1]
density:	0.5 g/cm³ [1]
melting point:	200°C[1]
vapor pressure:	negligible[1]
water solubility:	12 g/L (25°C)[1]
pKa (dissociation constants):	<2, 2.6, 5.6, 10.6 [3]
K_{oc} (soil adsorption coeff.):	2640 [4]
proportion of comm.form.:	41.0% (isopropylamine salt)[2]
concentration free acid in commercial formulation:	356 g/L [2]
concentration of salt in commercial formulation:	480 g/L [2]
surfactant in commercial formulation:	polyethoxylated tallow amines
pH of commercial formulation:	4.8 [2]
specific gravity of commercial formulation:	1.17 [2]

Sources: [1] WSSA 1983; [2] Monsanto 1982; [3] Sprankle et al. 1975b
[4] Kenaga & Goring 1980

Evaluation of the effects of glyphosate on aquatic flora have been varied. Richardson et al. (1979) found that glyphosate only inhibited cell density and chlorophyll content of a culture of Euglena gracilis at concentrations greater than or equal to 1.2×10^{-3} (ca. 200 mg/L), while stimulation of oxygen evolution was noted at 6×10^{-4} (100 mg/L). Conversely, Christy et al. (1981) found that growth of a laboratory culture of Chlorella sorokiniana was decreased slightly by treatment with 5.9×10^{-6} and 11.8×10^{-6} M (1 and 2 mg/L respectively) glyphosate, with increasing inhibition at 4.4×10^{-5} M and complete inhibition at all concentrations from 23.7×10^{-5} to 591×10^{-5} (100 mg/L). The inhibition could be reduced by addition of kaolinite clay, which presumably acted to absorb glyphosate and thereby render it phytotoxically inactive. Hartman & Martin (1984) reported a 2 mg/L glyphosate ED_{50} to Lemna minor (common duckweed), whereas concentrations of up to 10 mg/L had no inhibitory effects when suspended solids were added to the culture medium. Chan & Leung (1986) reported a reduction in bacterial biomass (mixed community) of a small pond treated with 200 mg/L glyphosate, although the inhibitory effect lasted only about 6 days. In aquatic macrophyte taxa, significant basipetal translocation of glyphosate taken up from surrounding water has been demonstrated for Potamogeton pectinatus (Marquis et al. 1981), although exposures of up to 0.32 mg/L were non-toxic to Elodea canadensis, Myriophyllum spicatum and Lemna minor (Forney & Davis 1981) and 10 mg/L glyphosate had no apparent effect on the growth of Potamogeton pectinatus (Hartman & Martin 1985).

To assess the potential effects of herbicide usage in forested areas of Manitoba on the primary productivity of adjacent aquatic ecosystems, a three-year project was initiated by the Water Standards and Studies Section of the Manitoba Department of Environment and Workplace Safety and Health under the terms of the Canada-Manitoba Forest Renewal Agreement. Glyphosate was chosen for aquatic toxicological evaluation because of its potential widespread use in Manitoba. The rationale and experimental design are described by Goldsborough & Brown (1986a).

A major proportion of total primary production in shallow ponds may be contributed by benthic algae (Wetzel 1983). Evaluations of the effects of the herbicides simazine and terbutryn (Goldsborough & Robinson 1983; 1986; 1987), atrazine (Herman et al. 1986), hexazinone (Anderson 1981), and diquat and endothall (Hodgson & Carter 1982) have shown that structural or functional changes in these communities may occur. Sullivan et al. (1981) studied effects of a 2.2 L/ha Roundup application on the density of some periphytic diatoms, but could not differentiate toxicological impact from natural seasonal succession. These authors did not quantify changes in algal productivity. The objective of the present study was to assess the effects of the Roundup formulation of glyphosate on short-term carbon fixation by periphytic algae in each of the six experimental study ponds described by Goldsborough & Brown (1986a,b).

2.0 Materials & Methods

The study ponds are located on the western edge of the Canadian Precambrian Shield bordering Lake Winnipeg, 12 to 25 kilometers north of the Pine Falls Hydroelectric Generating Station (ca. 96° 10'W, 50° 37'N). The pond basins were constructed approximately 28 years ago as a consequence of borrow pit excavation during highway construction, and have since filled with water, become surrounded by woody and herbaceous vegetation, and colonized by both nonvascular and vascular plant taxa. Chemical and physical characterizations of the ponds have been performed (Goldsborough & Brown 1986b) and measurements of primary production by periphytic and planktonic algae, and aquatic macrophytes have been made at periodic intervals throughout the ice-free season (Goldsborough & Brown unpubl.).

A synopsis of pondwater chemistry during the period of this study is given in Table 2. Concentrations of major cations (calcium, magnesium and sodium) and bicarbonate alkalinity were consistently higher in Spruce Pond than in all other ponds throughout the summer and fall (Goldsborough & Brown 1986b). Raspberry, Pine and Birch Ponds were dystrophic (Secchi disk depth 0.43 to 2.00 m, true color 35 to >50 Pt units) while the other three ponds were comparatively less colored (Secchi depth 1.35 to >3.18 m, color <5 to 20 Pt units). The surface water temperature at the time of sample collection was about 4°C, and ice crusts were observed in early morning at all sites.

Extruded acrylic rods (Goldsborough et al. 1986) were used as artificial substrata for periphytic algal colonization. Due to the high gravel content of the pond bottoms, the substrata could not be supported in the sediments. Instead, 10-12 substrata were imbedded vertically in a flat concrete base (5cm x 30cm x 30cm) which in turn was laid on the sediment surface. The pattern of substratum scoring permitted the collection of four 2.5 cm segments at the 10-20 cm position on each rod (height above base), and a 10 cm segment from the 20-30 cm position. Substrata were positioned in each pond on 26 August, 1985 (water depth 41-46 cm; Figure 1) and allowed to colonize with algae for a period of 44-52 days (Table 3). Two separate sets of substrata (two bases) were positioned at one site in Birch Pond (Figure 1) to provide algal material for a preliminary experiment, in which the appropriate range of herbicide concentrations for further testing was determined.

The first collection of substrata from Birch Pond was made on 8 October, 1985. Four 2.5cm rod segments were sampled from the 10-17.5 cm position from 10 rods and placed into clear glass tubes containing 20 mL filtered (Whatman GF/C) pondwater. The remaining upper 10 cm segment was placed into an empty tube for subsequent chlorophyll analysis. All tubes were transported to the laboratory and allowed to reach the incubation temperature (16°C) before commencement of herbicide and radioisotope additions. Samples for chlorophyll analyses were immediately frozen. Serial aqueous dilutions of Roundup (35.6% glyphosate acid formu-

Table 2: Surface water chemistry at the time of periphyton sample collection from the six study ponds (8-17 Oct. 1985).

Parameter	Spruce	Raspberry	Pine	Golden	Hike	Birch
conductivity (uS/cm)	463	138	57.3	91.0	111	51.0
pH	8.47	6.81	6.48	7.10	7.44	6.70
total alkalinity (mg/L)	232	56.0	24.0	45.0	55.0	24.0
TIC (mg/L)	56.5	18.6	9.6	13.4	14.4	8.9
DOC (mg/L)	21.6	67.0	27.0	10.4	9.9	18.6
calcium (mg/L)	33.0	17.3	6.97	13.0	12.9	5.91
magnesium (mg/L)	33.3	10.6	3.32	4.08	5.42	2.92
manganese (mg/L)	<0.02	0.09	0.19	0.04	0.06	0.04
iron (mg/L)	0.02	1.12	2.61	0.21	0.44	4.12
potassium (mg/L)	<5.00	<5.00	<5.00	<5.00	<5.00	<5.00
sodium (mg/L)	39.6	6.49	1.60	1.30	3.74	1.80
chloride (mg/L)	<2.0	3.0	<2.0	<2.0	<2.0	<2.0
fluoride (mg/L)	0.27	0.13	<0.1	<0.1	0.11	<0.1
sulphate (mg/L)	28	14.1	4.9	1.1	1.2	4.7
TKN (mg/L)	0.95	1.9	1.1	0.65	0.60	0.70
ammonia-N (mg/L)	<0.02	0.03	<0.02	<0.02	<0.02	<0.02
NO_3/NO_2-N (mg/L)	0.03	<0.01	<0.01	<0.01	<0.01	<0.01
total phosphorus (mg/L)	0.015	0.033	0.053	0.040	0.018	0.018
silica (mg/L)	1.8	9.5	4.7	1.2	2.1	2.7

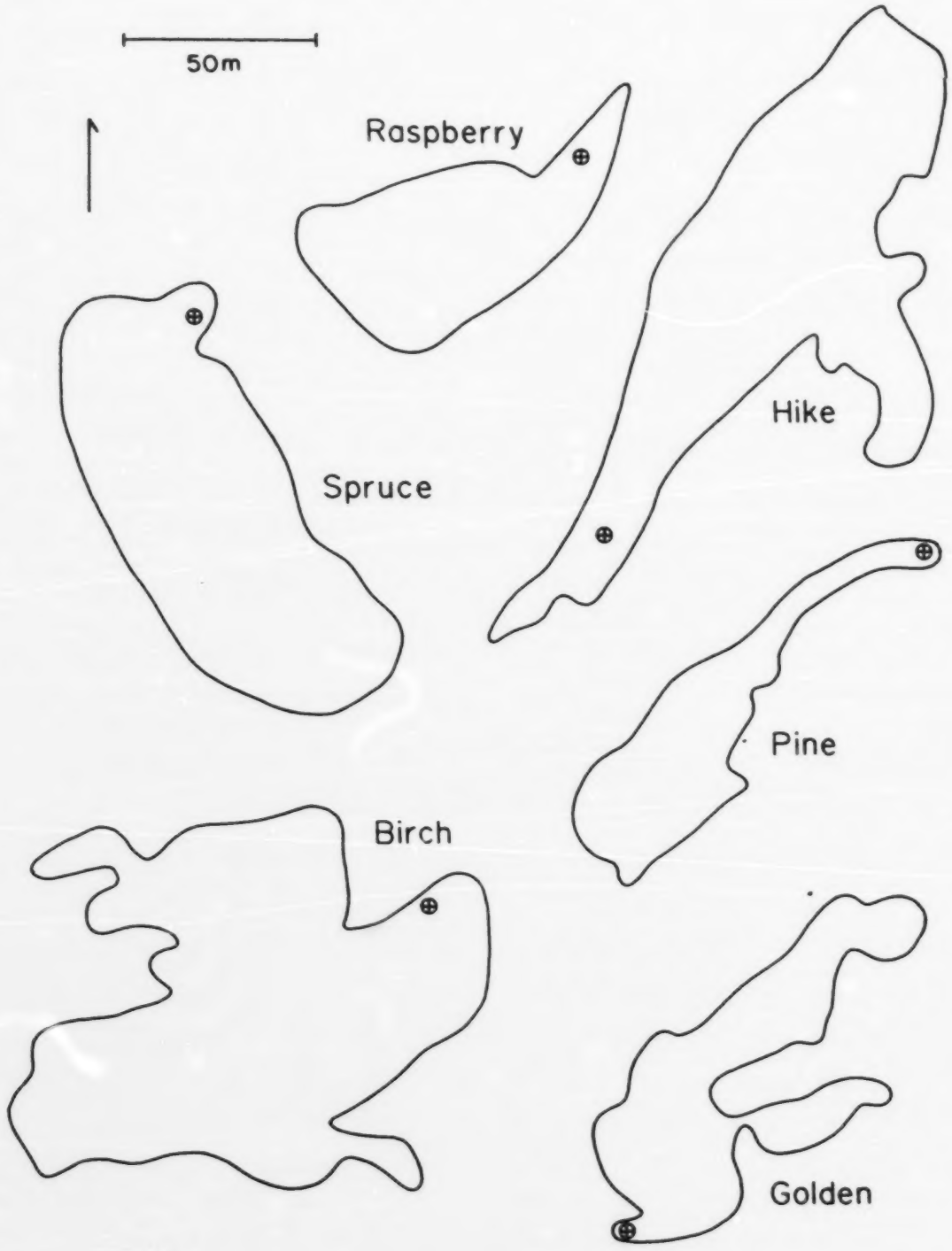


Figure 1: Locations (⊕) of artificial substrata sampling stations in the six study ponds, Pine Falls, 1985. Two sets of substrata were positioned at the Birch Pond station. Ponds are not shown in their correct geographic locations.

lated as an isopropylamine salt) were prepared such that 0.1 mL aliquots added to the incubation media yielded glyphosate concentrations of 8.9×10^{-6} to 8.9×10^{2} mg/L (5.3×10^{-11} to 5.3×10^{-3} M). Herbicide treatments and one distilled water control treatment were assigned to samples according to a randomized incomplete block design. One half millilitre of $NaH^{14}CO_3$ (18.5 kBq/mL) was added to each tube, and the contents were mixed thoroughly. All samples were incubated in a controlled environment chamber at a light intensity of 200 umoles/m^2/s for four hours with intermittent agitation. Substratum segments and any dislodged periphyton were then collected onto 0.45 um filters under vacuum and post-washed with 10 mL distilled water. The samples were fumed over concentrated HCl to liberate residual inorganic ^{14}C, and placed in scintillation vials containing 5 mL Scintiverse cocktail. Radioactivity was determined using a Picker Liquimat 220 scintillation counter with quench correction based on the channels ratio method (Wang & Willis 1965). Total inorganic carbon (TIC) in the incubation media was estimated from titratable alkalinity (Goldsborough & Brown 1986b), pH and incubation temperature, and used to calculate absolute carbon fixation rates (ug C/cm^2/h) of periphyton samples.

Based on the findings of the preliminary experiment, herbicide treatments of less than 0.89 mg/L were excluded from further experiments. The glyphosate concentrations used for bioassays of Spruce and Raspberry Pond samples were higher than originally intended due to a calculation error, and included final concentrations of 0.89, 8.9, 89, 180, 360, 530, 710, 890 and 1800 mg/L glyphosate. The concentrations used in all other bioassays were 0.89, 8.9, 18, 36, 53, 71, 89, 890 and 1800 mg/L. Substrata were sampled on 11 October, 1985 (Spruce and Raspberry Ponds), 15 October, 1985 (Pine and Golden Ponds) and 17 October, 1985 (Hike and Birch Ponds) according to the above protocol. Three replicate samples were used at each herbicide concentration and a 12.5 cm segment was collected for chlorophyll analyses.

The radioactivities of samples treated with glyphosate were expressed as a percentage of the control treatment and subtracted from 100 to derive estimates of relative inhibition at each glyphosate concentration. These data and $\log_{10}$-transformed herbicide concentrations were fitted to the probit function (Hubert 1980) using a modified version of the BASIC computer program of Trevors & Lusty (1985). A chi-squared goodness of fit test was used to test a null hypothesis that the fitted probit line adequately modelled that data (using a critical chi-squared value at 6 d.f. and 95% confidence of 12.59), after which EC_{50} (glyphosate concentration resulting in 50% inhibition of carbon fixation) and 95% confidence limits were calculated (Trevors & Lusty 1985).

Chlorophyll was extracted directly from thawed substratum segments by immersion in 10 mL neutralized 90% methanol for a period of 24 hours in the dark. Details of the spectrophotometric and calculation methods employed are described in Goldsborough & Brown (1986a), and data were expressed as total chlorophyll (chlorophyll *a* plus its degradation product, pheophytin *a*).

3.0 Results

There were clear differences in the algal colonization of substrata from each pond (Table 3). On bases of total chlorophyll content and laboratory carbon-fixation rates, Pine Pond substratum samples were most heavily colonized, while Spruce Pond samples were least. Algal biomass on substrata from Raspberry, Golden and Hike Ponds, and preliminary samples from Birch Pond were similar, while the second series of samples from Birch Pond supported less biomass than the first (Table 3).

Carbon fixation rates of samples treated with 8.9×10^{-6} to 8.9 mg/L glyphosate were similar to those of control samples (Figure 2) in the preliminary experiment conducted using Birch Pond samples. Rates were 20% and 14% of the control at 89 mg/L and 890 mg/L respectively. On the basis of these data, it seems reasonable to extrapolate that glyphosate concentrations less than 0.89 mg/L have no effect on short-term algal photosynthesis, while a 50% effective dose (EC_{50}) must lie between 8.9 and 89 mg/L. Further testing therefore was focussed between these concentrations. A 1800 mg/L treatment, the highest level attainable under the present experimental design, was also added.

Glyphosate concentrations less than 0.89 mg/L had no effect on carbon fixation rate of periphyton samples from Spruce (Figure 3) and Raspberry (Figure 4) Ponds. Fixation rates of Spruce Pond samples treated with 180 mg/L glyphosate amounted to 20% of the control. The levels of inhibition decreased (Figure 3) to approximately 67% of the control at 1800 mg/L. The low replicate variability of these treatments suggests that the trend is real. A more typical result was found in Raspberry Pond (Figure 4). The degree of inhibition at 89 and 890 mg/L was less than corresponding data for Spruce Pond but all higher concentrations resulted in greater inhibition. Detectable carbon fixation occurred at 1800 mg/L glyphosate in Raspberry Pond (15% of control). Due to the clear departure of Spruce Pond samples from a typical dose-response curve, the probit line fitted to these data was not significant at the 5% level (chi-square = 93.26). An EC_{50} value may not therefore be inferred. The abrupt decrease in carbon fixation by Raspberry Pond samples at 89 mg/L glyphosate was due to the lack of treatments between 8.9 and 89 mg/L, with the result that the corresponding probit line was also not significant (chi-squared = 48.28). However, visual inspection of the data indicates that an EC_{50} would lie between 8.9 and 89 mg/L (Figure 4).

Carbon fixation of Pine Pond samples decreased in relation to the control at glyphosate concentrations greater than 0.89 mg/L (Figure 5). The range of inhibition varied from 76 ± 10% at 8.9 mg/L to 24 ± 4% of the control at 1800 mg/L. The fitted probit line was statistically significant (chi-squared=2.15) and provided an EC_{50} estimate of 69.7 mg/L (95% confidence limits 50.7, 98.5 mg/L).

Table 3: Estimated biomass (mean ± SD, n=10) of periphytic algae on artificial substrata (17.5-30 cm above base) collected from study ponds, based on total chlorophyll *a* concentration and carbon fixation rate (4-hour incubation; 8-17 Oct. 1985). A = preliminary experiment.

Pond	Colonization time (d)	Total chlorophyll *a* (ug/cm^2)	Absolute carbon fixation rate ($ug/cm^2/hr$)
Spruce	46	0.02 ± 0.01	0.15 ± 0.10
Raspberry	46	0.19 ± 0.04	0.68 ± 0.13
Pine	49	0.26 ± 0.05	1.12 ± 0.14
Golden	49	0.14 ± 0.04	0.45 ± 0.17
Hike	52	0.14 ± 0.07	0.68 ± 0.21
Birch (A)	44	0.14 ± 0.02	0.88 ± 0.29
(B)	52	0.07 ± 0.04	0.70 ± 0.18

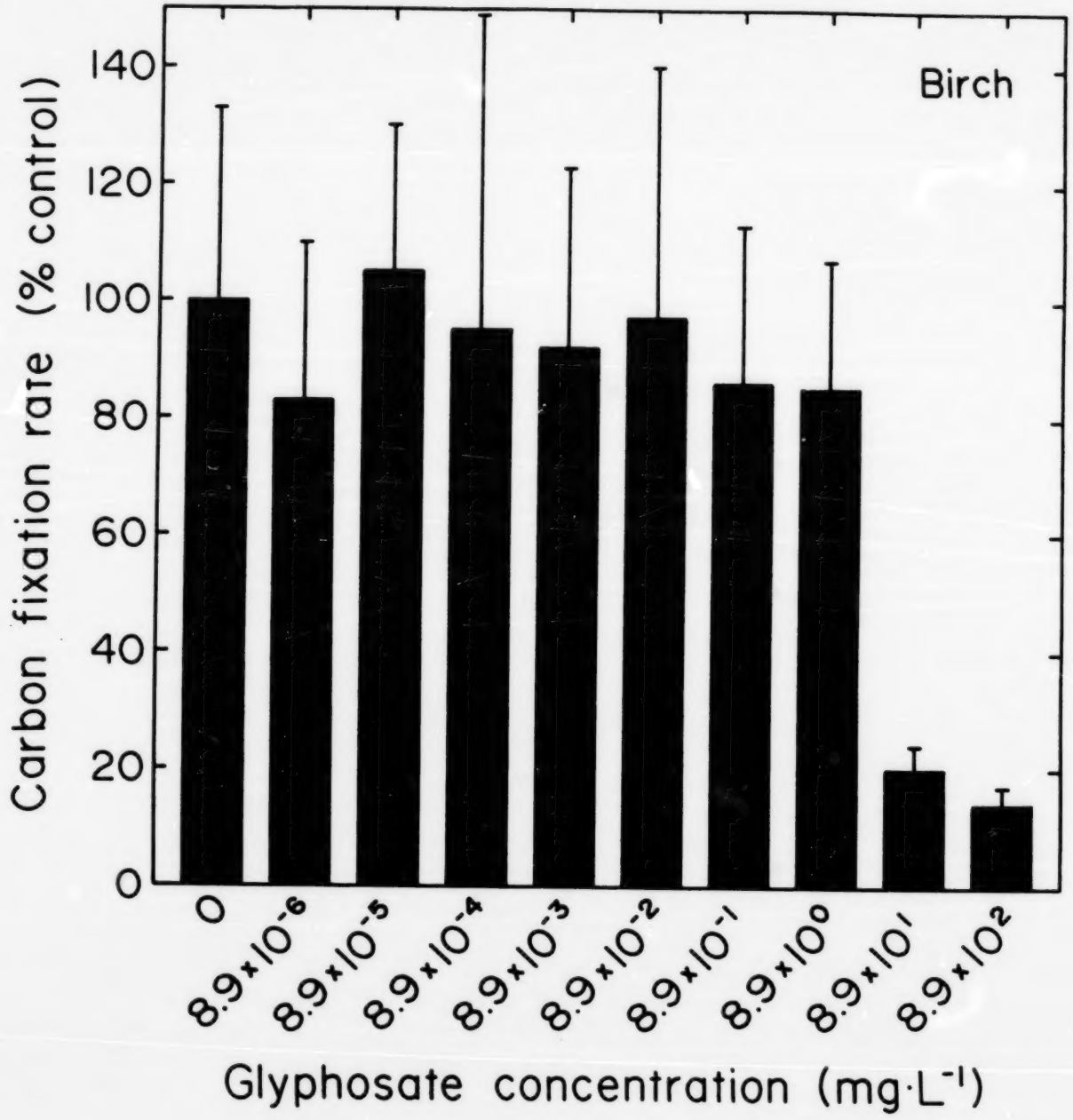


Figure 2: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Birch Pond on 8 October, 1985.

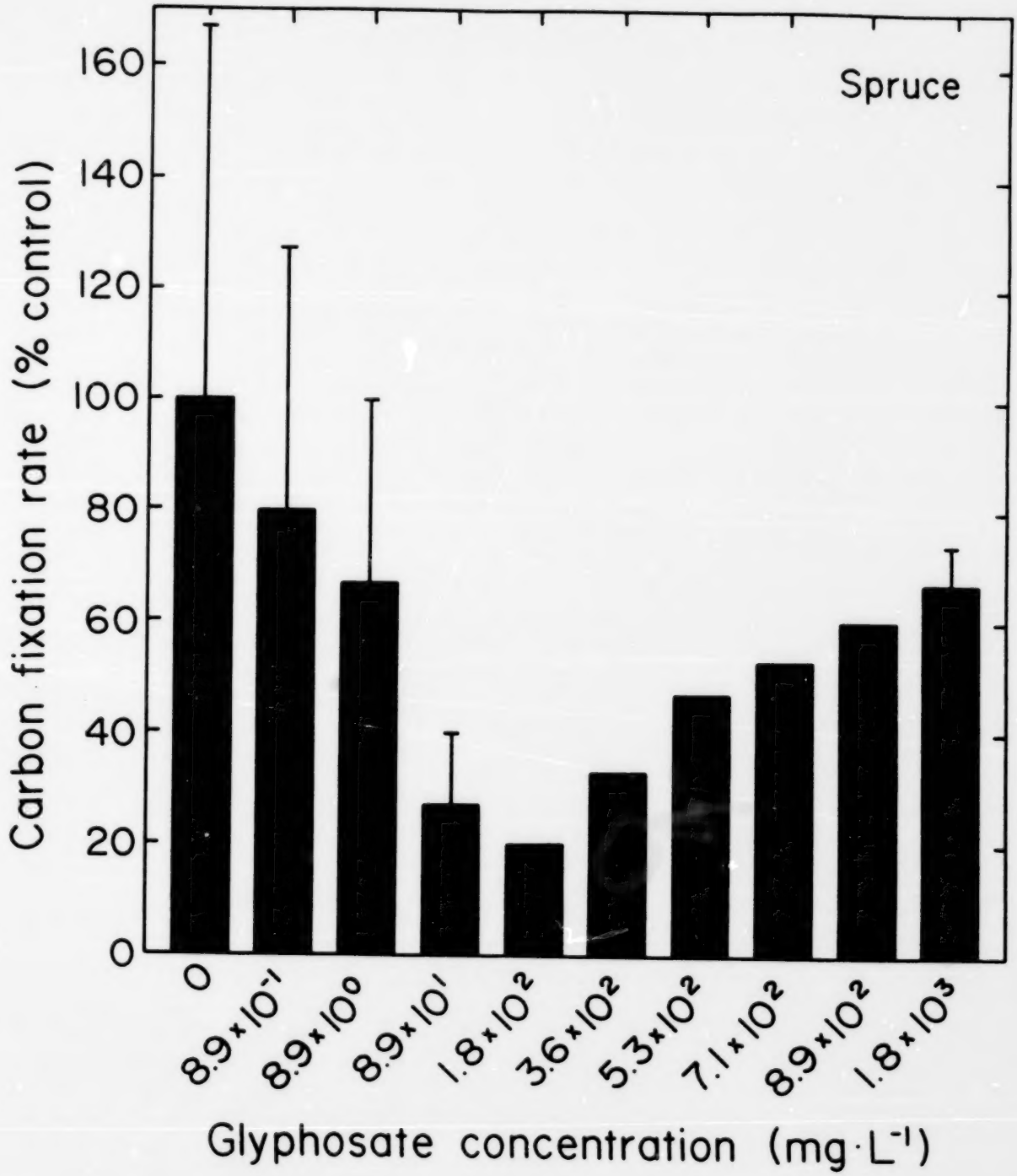


Figure 3: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Spruce Pond on 11 October, 1985.

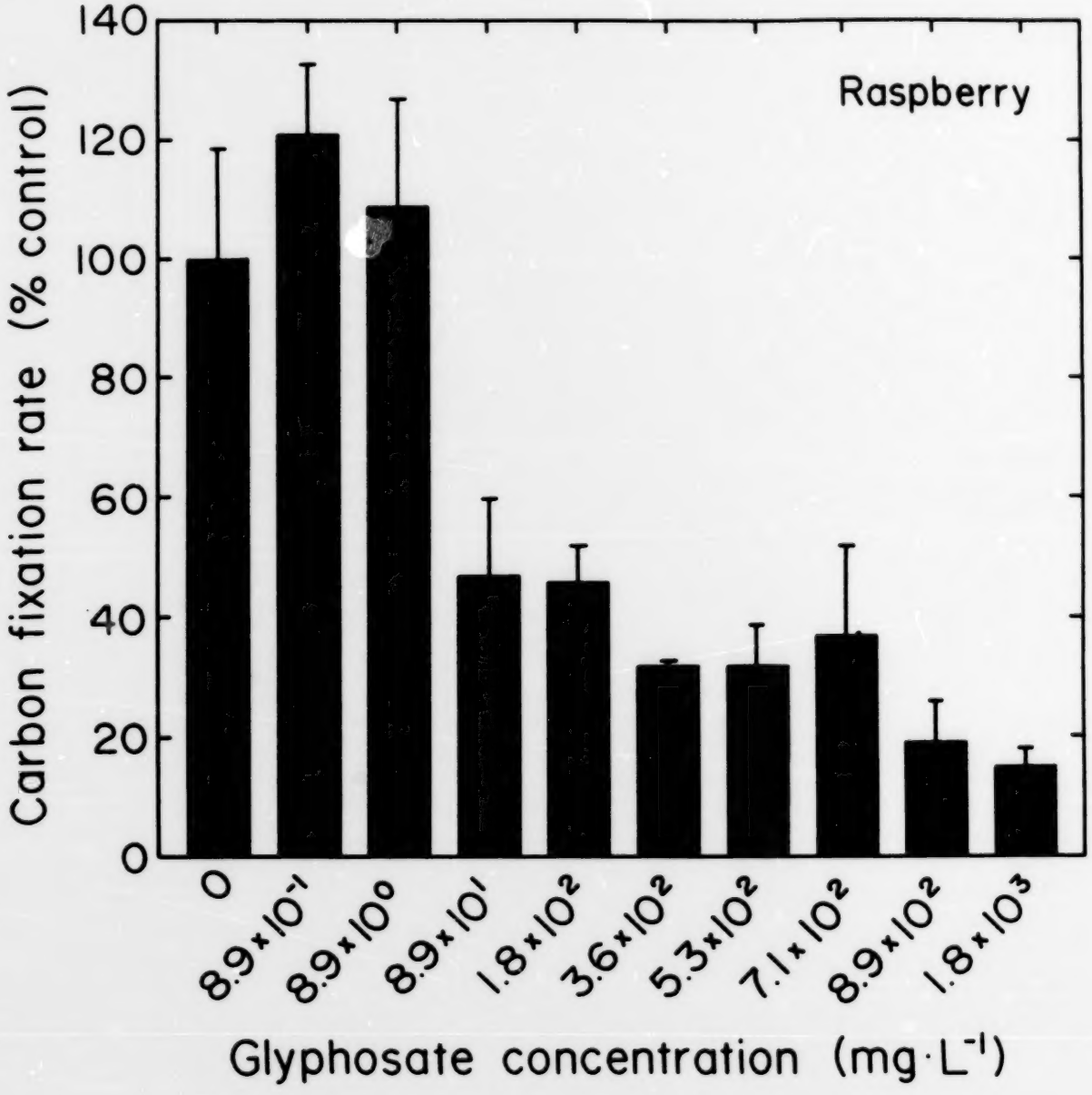


Figure 4: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Raspberry Pond on 11 October, 1985.

Replicate variability of samples from Golden Pond was high (coefficient of variability 17-77%) with the result that effects of treatments from 0.89 to 71 mg/L could not be distinguished from the control (Figure 6). There was a general decrease in mean carbon fixation in this range, however, and marked decreases occurred from 89 to 1800 mg/L (22-27% of control). The fitted probit line was not significant (chi-squared = 255.86), but visual interpolation of the data pointed to an EC_{50} value between 18 and 89 mg/L glyphosate (Figure 6).

The response of periphyton from Hike Pond to glyphosate (Figure 7) was similar to that from Pine Pond. The 0.89 mg/L treatment did not cause detectable decreases in carbon fixation, with reductions at all higher concentrations to a level of 25±6% of the control at 1800 mg/L. The fitted probit line was marginally significant at the 5% level (chi-squared = 12.58). The estimated EC_{50} value was 44.4 mg/L (95% confidence limits 33.4, 60.5 mg/L).

With the exception of a 89 mg/L glyphosate treatment, carbon fixation rates of periphyton samples from Birch Pond varied inversely with herbicide concentration (Figure 8). At 1800 mg/L glyphosate, carbon fixation was 11±3% of untreated control samples. The estimated EC_{50} value was 35.4 mg/L with 95% confidence limits of 29.2 and 43.5 mg/L (chi-squared = 11.50).

4.0 Discussion

Van Rensen (1974) concluded on the basis of the response of DCPIP dye reduction in isolate spinach chloroplasts to glyphosate that glyphosate directly inhibits photosynthetic electron transport in the vicinity of photosystem II. After a 60-90 minute exposure to 7×10^{-4} M (120 mg/L) glyphosate, oxygen evolution by a culture of the green alga Scenedesmus sp. decreased by 50%. Richard et al. (1979) reexamined these results in light of experiments using pea chloroplasts, which showed no effects on photosynthetic activity. They theorized that any such effects may be due to changes in ambient pH rather than direct phytotoxicity of the herbicide. Glyphosate is known to be a weak acid (Table 1) and can release hydrogen ions (Sprankle et al. 1975b), causing the pH of weakly buffered media to decrease (Richard et al. 1979). However, this effect may be restricted to the pure reagent, since the commercial formulation of glyphosate as an isopropylamine salt likely buffers any change in pH (D.C.G.Muir, pers.comm. 1987).

The short-term photosynthetic rates of intact periphyton communities, as measured by gross inorganic carbon assimilation, were significantly impacted by glyphosate additions. Except for Spruce Pond data, there was general agreement in the level of toxicity found for algal communities from each of the study ponds. A threshold glyphosate concentration, below which photosynthesis was not sigificantly different from untreated control samples, was approximately 0.89 mg/L in Pine and Hike Ponds, and

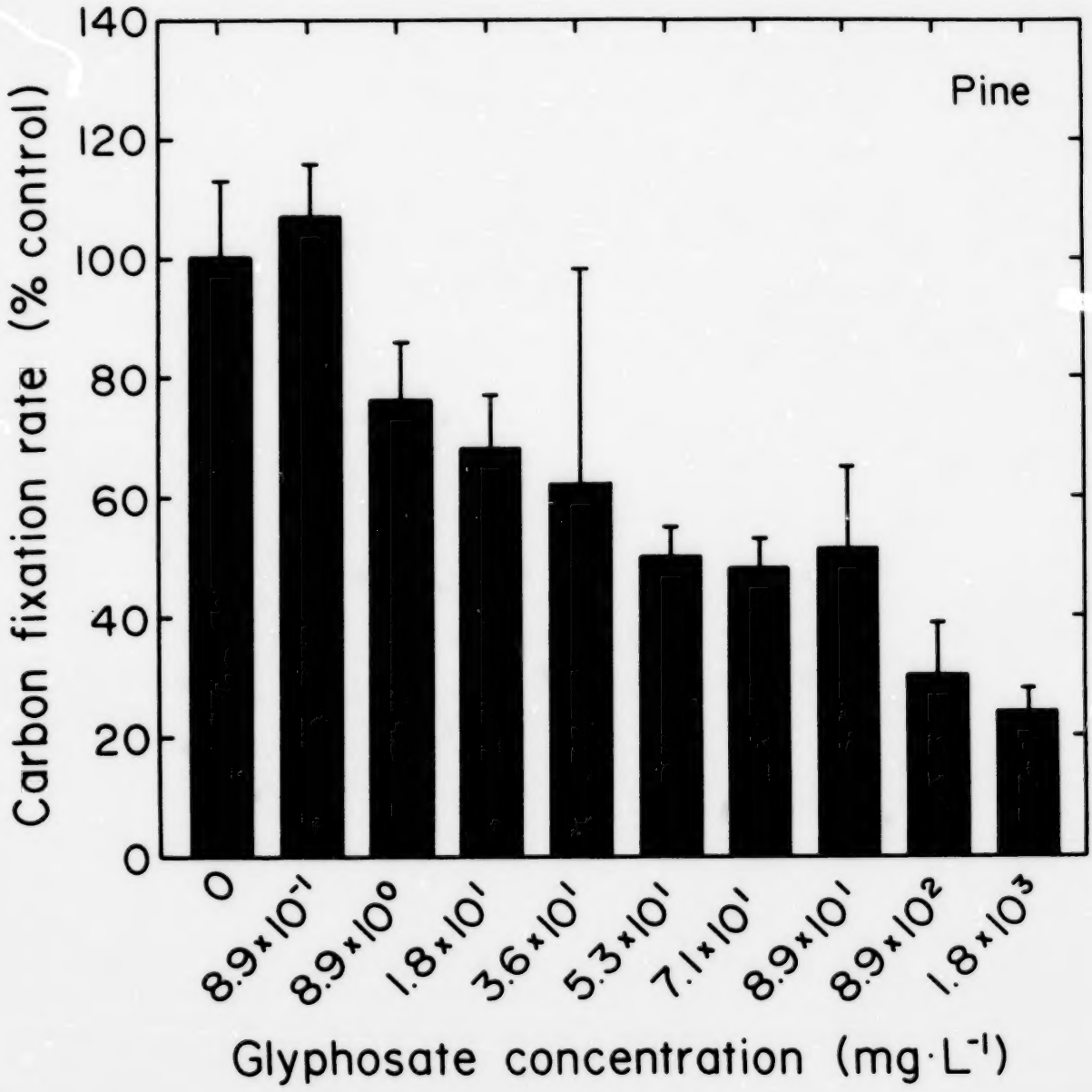


Figure 5: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Pine Pond on 14 October, 1985.

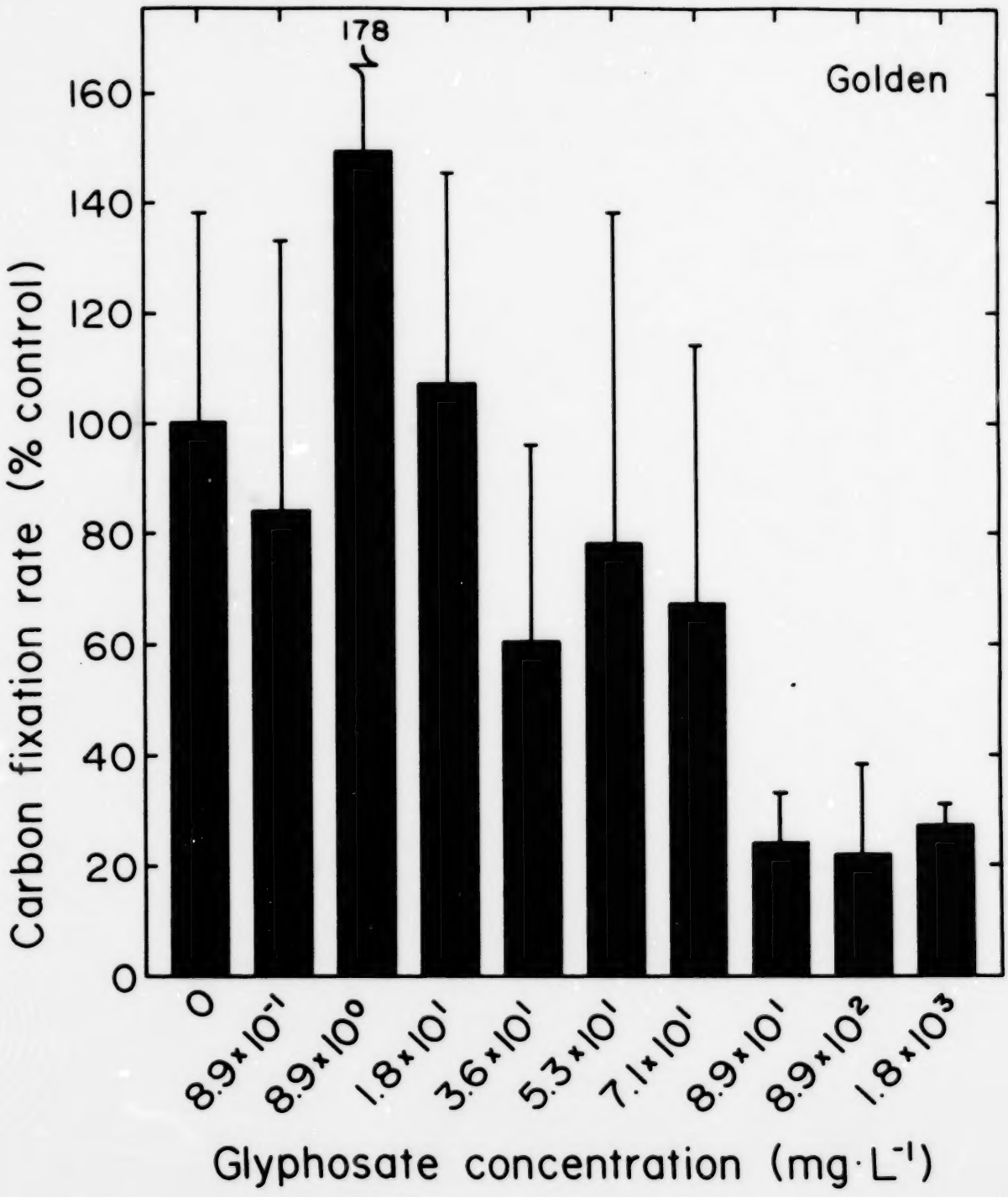


Figure 6: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Golden Pond on 14 October, 1985.

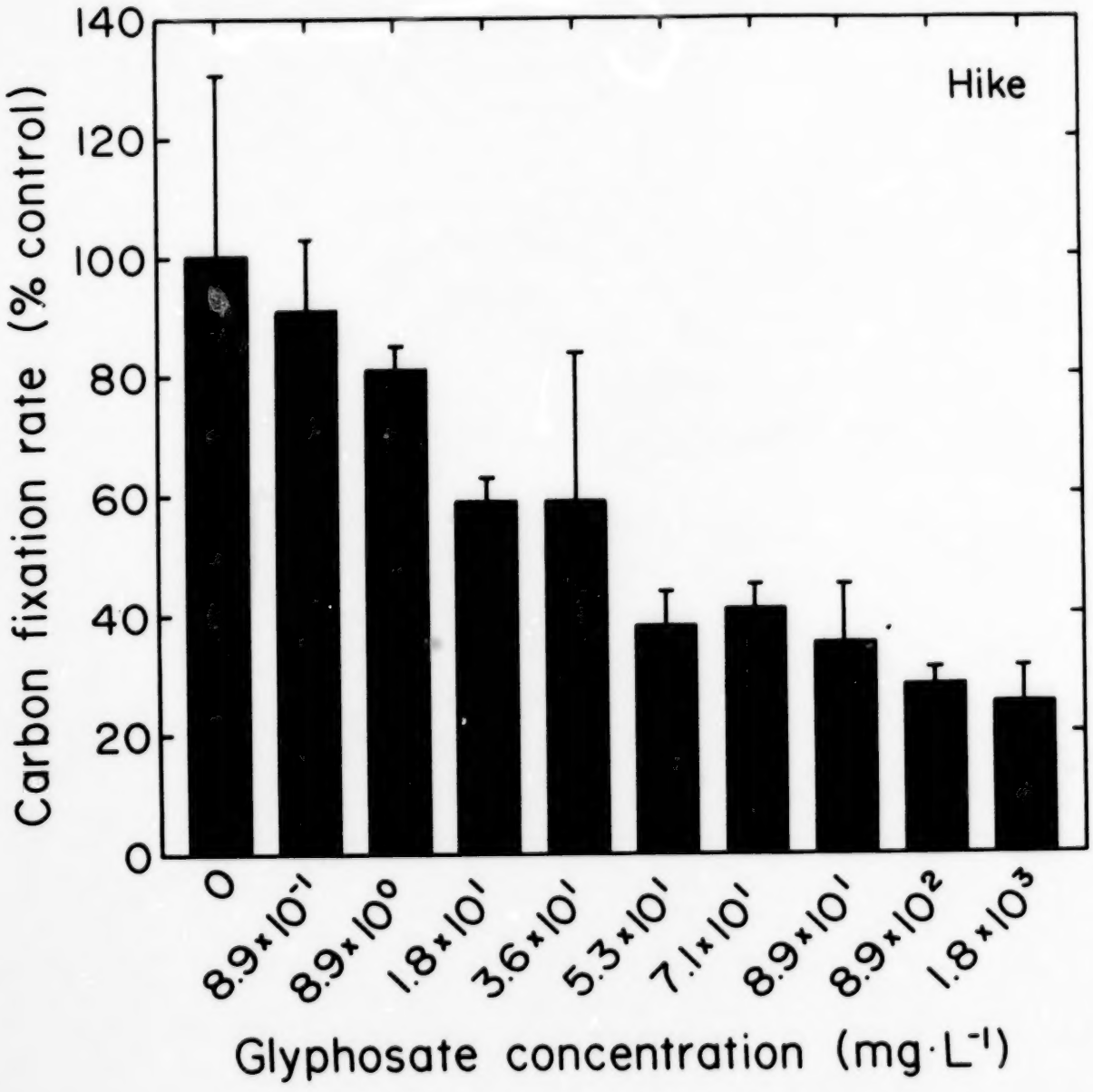


Figure 7: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Hike Pond on 17 October, 1985.

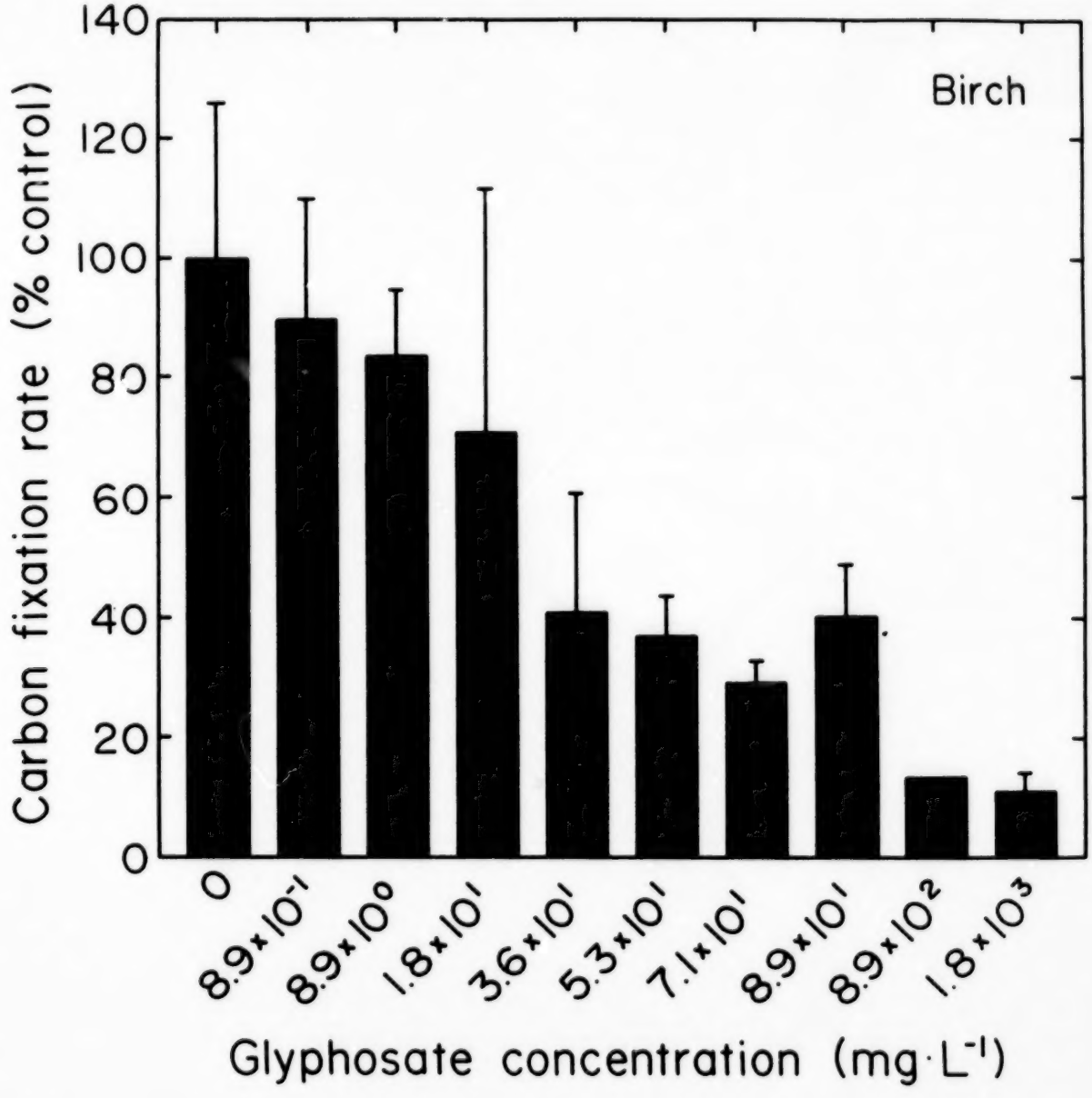


Figure 8: Response of 4-hour periphytic algal carbon fixation rate (% unmanipulated control) to laboratory additions of glyphosate (Roundup). Samples were collected from Birch Pond on 17 October, 1985.

8.9 mg/L in Birch Pond (preliminary experiment), Raspberry and Golden Ponds. Between 8.9 and 89 mg/L, photosynthetic activity decreased consistently with increasing herbicide concentration. Carbon fixation was not completely inhibited at the highest glyphosate concentration used in these experiments (1800 mg/L) where rates amounting to 11-27% of the control were observed.

If glyphosate inhibits photosynthetic electron transport directly, then it is clearly less effective than other known photosynthetic inhibitors. The triazine herbicides, including the forestry herbicide hexazinone, are potent photosystem II inhibitors (Moreland 1980) and have been shown to inhibit short-term photosynthesis and growth of periphytic algae. For example, the EC_{50} to photosynthesis of the triazine herbicide simazine was about 0.8 mg/L for a community made up largely of freshwater diatoms (Goldsborough & Robinson 1987). Despite considerations of community specificity of EC_{50} values (Goldsborough & Robinson 1987), glyphosate is either less effectively bound to the herbicide-binding site in photosystem II, is largely excluded from the chloroplast, or observed changes in photosynthetic rate were derived from secondary effects of herbicide addition.

The suggestion of Richard et al. (1979) that glyphosate bioassays employ pH adjustment to counteract acidification of culture media may be justified in the interest of standardization but is environmentally unrealistic on the grounds that pH modification, if it indeed occurs using the commercial formulation, would accompany field applications to water. It is felt that the present results, which are based on the use of natural pond waters of varying chemical composition (Table 2), are more realistic evaluations of probable <u>in situ</u> effects on photosynthesis. However, these data do not indicate whether the reduction in photosynthetic rate noted at glyphosate concentrations greater than 8.9 mg/L were due to changes in pH, primary herbicide toxicity, or secondary toxicity of another component in the Roundup formulation. The ponds studied here have generally low alkalinity and pH (Goldsborough & Brown 1986b; Table 2) and so would be poorly buffered against acid input. Of the six ponds, Spruce Pond is most alkaline and therefore least likely to become acidified. It is also the only pond of those studied which did not respond in a consistent way to glyphosate additions (Figure 3).

The responses of periphytic algal photosynthesis were expressed here in terms of the concentration of glyphosate rather than the Roundup formulation used in experimentation. However, these results may not be directly comparable to studies using pure glyphosate. The chemical composition of Roundup is not in the public domain, but if generally similar to other herbicides, consists of glyphosate (formulated as its isopropylamine salt to increase water solubility), and a surfactant (reportedly a polyethoxylated tallow amine) to enhance adhesion to plant surfaces. In tests with various aquatic fauna, Folmar et al. (1979; summarized by Ghassemi et al. 1981) found that the surfactant component alone was more toxic than glyphosate, suggesting that the efficacy of Roundup should be greater than that of glyphosate. If

this generalization applies to phytotoxic effects, the present findings would represent overestimates of the impact of glyphosate but would be more realistic of natural Roundup usage. The apparently spurious results of the Spruce Pond bioassay may represent the outcome of an interaction between pondwater chemistry and a component of the Roundup formulation, altering the effective herbicide concentration at higher treatment levels.

Hance (1976) has suggested that glyphosate binds competitively with phosphate in soils, but it is not known whether the phosphorus moiety in glyphosate can act as a direct functional replacement for orthophosate in plant metabolism, or if plants can metabolize it as a phosphorus source. Peverly & Crawford (1975) speculated that glyphosate could act as a phosphorus fertilizer and that this could ameliorate phytotoxicity. Calculations based on the proportion of phosphorus in the glyphosate molecule indicate that phosphate concentrations as high as 1000 mg/L could be achieved at 1800 mg/L glyphosate. Given that dissolved and total orthophosphate levels were below detection limits in all study ponds throughout the summer (Goldsborough & Brown 1986b), and total phosphorus levels were also low (Table 2), such inputs could significantly affect _in situ_ growth and perhaps photosynthetic rates during a four hour incubation. The possibility that glyphosate is directly and rapidly decomposed to an inorganic form usable to plants seems remote, however, since orthophosphate has not been reported as a common metabolite of glyphosate in water under normal environmental conditions (Sacher 1978) and reported decomposition half lives in soil/water systems are at least 38 days (Rao & Davidson 1980).

Intact periphyton communities consist of a complex assemblage of organisms differing in their innate sensitivity to glyphosate, so there is no _a priori_ reason why EC_{50} values should be the same for samples from each of the ponds. Of the three ponds for which EC_{50} could reliably be calculated, overlap of 95% confidence intervals occurred between Pine and Hike Ponds, and between Hike and Birch Ponds. The EC_{50} limits of Pine Pond samples did not overlap those of Birch Pond. These differences cannot be interpreted statistically in terms of interpond variability, however, because intrapond variability was unquantified (Hurlbert 1984). Although algal species composition of substratum samples was not determined, marked differences in the gross appearance of samples from various ponds were noted. For example, samples from Pine Pond were dark brown and covered the substrata uniformly, whereas samples from Hike Pond supported heterogeneous patches of globular cyanobacteria and green algae. Moreover, the extent of substratum colonization in each pond (Table 3) may have a bearing on sensitivity to herbicide. Pine Pond samples, which had the highest EC_{50} value of the three ponds, supported the greatest algal biomass, while biomass estimates and EC_{50}'s of Hike and Birch Pond samples were both lower.

The glyphosate concentration in pondwater which could result from direct application of Roundup to the surface of the study ponds was estimated in order to place the present results in

context of potential *in situ* effects of glyphosate on algal photosynthesis. Based on a typical Roundup application rate of 2.5 L/ha (89 kg a.i./ha) at reforested areas near the study site (A.Russell, Abitibi-Price Ltd, pers.comm. 1986), pond morphometry (Table 4 - from Goldsborough & Brown 1986b), and assuming that surface deposition is homogeneous, the herbicide is rapidly and uniformly mixed through the water column, and sediment sorptive and other losses are neglected, estimated concentrations of 0.06 to 0.10 mg/L are predicted (Table 4). Glyphosate concentrations tested in Great Britain for the control of aquatic weeds (Seddon 1981) were as high as 2.88 kg a.i/ha, which if applied to the study ponds, might lead to concentrations in pondwater of about 0.2 to 0.3 mg/L glyphosate. At these concentrations, no reduction in algal photosynthesis would be predicted on the basis of the present results (Figure 2-8). This does not preclude the occurrence of higher herbicide inputs due to multiple applications or a larger single dosage, nor is it implied that short-term effects on photosynthesis can be directly translated into net algal growth. It is emphasized that these preliminary findings relate only to responses of algal photosynthesis to glyphosate during a four hour exposure period, and may differ from longer term effects on growth occurring due to other effects of glyphosate on various aspects of cell metabolism. Field evaluation of long-term responses of algal productivity and biomass to glyphosate additions are currently underway.

Table 4: Hypothetical glyphosate concentration (mg/L) in water of the study ponds after a 2.5 L/ha Roundup application.

Pond	Surface Area (m^2)	Glyphosate Added (g)	Pond Volume (m^3)	Glyphosate Conc. (mg/L)
Spruce	5168	460	7347	0.06
Raspberry	2597	231	2374	0.10
Pine	1980	176	2130	0.08
Golden	2946	262	2802	0.09
Hike	6325	563	9152	0.06
Birch	7681	684	11389	0.06

5.0 Literature Cited

Anderson,L.W.J. 1981. Control of aquatic weeds with hexazinone. J.Aquat.Pl.Mgmt 19:9-14.

Bowmer,K.H., P.M.Boulton, D.L.Short & M.L.Higgins 1986. Glyphosate - sediment interactions and phytotoxicity in turbid water. Pestic.Sci. 17:79-88.

Chan,K. & S.C.Leung 1986. Effects of paraquat and glyphosate on growth, respiration, and enzyme activity of aquatic bacteria. Bull.Environ.Contam.Toxicol. 36:52-59.

Christy,S.L., E.P.Karlander & J.V.Parochetti 1981. Effects of glyphosate on the growth rate of Chlorella. Weed Science 29:5-7.

Comes,R.D., V.F.Bruns & A.D.Kelley 1976. Residues and persistence of glyphosate in irrigation water. Weed Science 24:47-50.

Folmar,L.C., H.O.Sanders & A.M.Julin 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. Arch.Environ.Contam.Toxicol. 8:269-278.

Forney,D.R. & D.E.Davis 1981. Effects of low concentrations of herbicides on submersed aquatic plants. Weed Science 29:677-685.

Ghassemi,M., L.Fargo, P.Painter, P.Painter, S.Quinlivan, R.Scofield & A.Takata 1981. Environmental fates and impacts of major forest use pesticides. U.S. Environmental Protection Agency Report EPA-68-01-3174, 484p.

Goldsborough,L.G. & D.J.Brown 1986a. Effects of aerial spraying of forestry herbicides on aquatic ecosystems. I. Procedures manual for project #803. Manitoba Department of Environment and Workplace Safety and Health, Water Standards and Studies, Report 86-6, 29pp.

Goldsborough,L.G. & D.J.Brown 1986b. Effects of aerial spraying of forestry herbicides on aquatic ecosystems. II. Baseline physical and chemical characterization. Manitoba Department of Environment and Workplace Safety and Health, Water Standards and Studies, Report 86-11, 131pp.

Goldsborough,L.G. & G.G.C.Robinson 1987. Functional responses of freshwater periphyton to short simazine exposures. Verh.Inter. Angew.Limnol. (in press).

Goldsborough,L.G. & G.G.C.Robinson 1986. Changes in periphytic algal community structure as a consequence of short herbicide exposures. Hydrobiologia 139:177-192.

Goldsborough,L.G. & G.G.C.Robinson 1983. The effect of two triazine herbicides on the productivity of freshwater marsh periphyton. Aquat.Toxicol. 4:95-112.

Goldsborough,L.G., G.G.C.Robinson & S.E.Gurney 1986. An enclosure/substratum system for in situ ecological studies of periphyton. Arch.Hydrobiol. 106(3):373-393.

Grossbard,E. & D.Atkinson (Eds.) 1985. The Herbicide Glyphosate. Butterworth, London.

Hance,R.J. 1976. Adsorption of glyphosate by soils. Pestic.Sci. 7:363-366.

Hartman,W.A. & D.B.Martin 1984. Effect of suspended bentonite clay on the acute toxicity of glyphosate to Daphnia pulex and Lemna minor. Bull.Environ.Contam.Toxicol. 33:355-361.

Hartman,W.A. & D.B.Martin 1985. Effects of four agricultural pesticides on Daphnia pulex, Lemna minor, and Potamogeton pectinatus. Bull.Environ.Contam.Toxicol. 35:646-651.

Herman,D., N.K.Kaushik & K.R.Solomon 1986. Impact of atrazine on periphyton in freshwater enclosures and some ecological consequences. Can.J.Fish.Aq.Sci. 43:1917-1925.

Hoagland,R.E. & S.O.Duke 1982. Biochemical effects of glyphosate [N-(phosphonomethyl)glycine]. In D.E.Moreland, J.B.St.John & F.D.Hess (Eds.) Biochemical Responses Induced by Herbicides. ACS Symposium Series 181, American Chemical Society, Washington, D.C. pp.175-205.

Hodgson,L.M. & C.C.Carter 1982. Effect of Hydrilla management by herbicides on a periphyton community. J.Aquat.Pl.Mgmt 20:17-19.

Hubert,J.J. 1980. Bioassay. Kendall/Hunt Publishing Co.,Dubuque, Iowa. 164pp.

Hurlbert,S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecol.Monogr. 54:187-211.

Jaworski,E.G. 1972. Mode of action of N-phosphonomethyl glycine: inhibition of aromatic amino acid biosynthesis. J.Agr.Fd Chem. 20:1195-1198.

Kenaga,E.E. & C.A.I.Goring 1980. Relationship between water solubility, soil sorption, octanol-water partitioning, and concentration in biota. In J.G.Eaton, P.R.Parrish & A.C.Hendricks (Eds.) Aquatic Toxicology, ASTM STP 707, American Society for Testing and Material. pp.78-115.

Marquis,L.Y., R.D.Comes & C.P.Yang 1981. Absorption and translocation of fluridone and glyphosate in submersed vascular plants. Weed Science 29:229-236.

Monsanto 1982. Monsanto Material Safety Data: Roundup Herbicide. Monsanto Co., St.Louis, Missouri. 4pp.

Moreland,D.E. 1980. Mechanisms of action of herbicides. Ann.Rev. Pl.Phys. 31:597-638.

Newton,M., K.M.Howard, B.R.Kelpsas, R.Danhaus, C.M.Lottman & S.Dubelman 1984. Fate of glyphosate in an Oregon forest ecosystem. J.Agric.Fd Chem. 32:1144-1151.

Peverly,J.H.& T.W.Crawford,Jr. 1975. Glyphosate as a herbicide for two submerged aquatic species. Proc.Northeast Weed Sci.Soc. 29:102.

Rao,P.S.C. & J.M.Davidson 1980. Estimation of pesticide retention and transformation parameters required in nonpoint source pollution models. In M.R.Overcash & J.M.Davidson (Eds.) Environmental Impact of Nonpoint Source Pollution. Ann Arbor Science Publishers Inc. pp.23-67.

Richard,E.P.,Jr., J.R.Gross & C.J.Arntzen 1979. Glyphosate does not inhibit photosynthetic electron transport and phosphorylation in pea (Pisum sativum) chloroplasts. Weed Science 27:684-688.

Richardson,J.T., R.E.Frans & R.E.Talbert 1979. Reactions of Euglena gracilis to fluometuron, MSMA, metribuzin, and glyphosate. Weed Science 27:619-624.

Sacher,R.M. 1978. Safety of Roundup in the aquatic environment. Proc. EWRS 5th Symp. Aquat. Weeds. pp.315-322.

Seddon,J.C. 1981. The control of aquatic weeds with the isopropylamine salt of N-phosphonomethyl glycine. Proc. Aquatic Weeds and their Control p.141-148.

Sprankle,P., W.F.Meggitt & D.Penner 1975a. Rapid inactivation of glyphosate in the soil. Weed Science 23:224-228.

Sprankle,P., W.F.Meggitt & D.Penner 1975b. Adsorption, mobility, and microbial degradation of glyphosate in the soil. Weed Science 23:229-234.

Sprankle,P., W.F.Meggitt & D.Penner 1975c. Absorption, action, and translocation of glyphosate. Weed Science 23:235-240.

Steinrucken,H.C. & N.Amrhein 1980. The herbicide glyphosate is a potent inhibitor of 5-enolpyruvyl-shikimic acid-3-phosphate synthase. Biochem.Biophys.Res.Comm. 94:1207-1212.

Sullivan,D.S., T.P.Sullivan & T.Bisalputra 1981. Effects of Roundup herbicide on diatom populations in the aquatic environment of a coastal forest. Bull.Environ.Contam.Toxicol. 26:91-96.

Trevors,J.T. & C.W.Lusty 1985. A BASIC microcomputer program for calculating LD_{50} values. Water,Air & Soil Poll. 24:431-442.

Van Rensen,J.J.S. 1974. Effects of N-(phosphonomethyl)glycine on photosynthetic reactions in Scenedesmus and in isolated spinach chloroplasts. In M.Avron (Ed.) Proceedings of the Third International Congress on Photosynthesis. Elsevier Publishing Co., Amsterdam. pp.683-687.

Wan,M.T.K. 1986. The persistence of glyphosate and its metabolite amino-methyl-phosphonic acid in some coastal British Columbia streams. Canada Dept.Environment, Conservation and Protection, Environmental Protection Service, Pacific and Yukon Region, Regional Program Report 85-01, 18p.

Wang,C.H. & D.L.Willis 1965. Radiotracer Methodology in Biological Science. Prentice-Hall, New York.

Weed Science Society of America (WSSA) 1983. Herbicide Handbook of the Weed Sciency Society of America. Champaign, Illinois.

Wetzel,R.G. 1983. Limnology, 2nd ed. Saunders College Publishing, Toronto.

Appendix A: Carbon fixation rates (ug/cm²/h) of periphyton samples (4 replicates) collected from Birch Pond (8 October, 1985) in response to glyphosate (Roundup) additions.

Glyphosate (mg/L)	Replicate 1	2	3	4
0	0.93	1.23	0.54	0.80
8.9×10^{-6}	0.44	0.96	0.89	0.61
8.9×10^{-5}	1.10	1.01	0.96	0.60
8.9×10^{-4}	0.90	0.48	1.49	0.49
8.9×10^{-3}	0.49	0.94	1.10	0.69
8.9×10^{-2}	0.96	1.33	0.67	0.44
8.9×10^{-1}	0.60	1.00	0.93	0.50
8.9	0.87	0.96	0.61	0.57
89	0.18	0.19	0.12	0.21
890	0.16	0.13	0.09	0.10

Appendix B: Carbon fixation rates (ug/cm²/h) of periphyton samples (3 replicates) collected from Spruce and Raspberry Ponds (11 October, 1985) in response to glyphosate (Roundup) additions.

Glyphosate (mg/L)	Spruce 1	2	3	Raspberry 1	2	3
0	0.14	0.25	0.06	0.53	0.79	0.71
0.89	0.08	0.08	0.20	0.89	0.74	0.84
8.9	0.09	0.06	0.16	0.67	0.67	0.87
89	0.02	0.06	0.03	0.23	0.33	0.40
180	0.03	0.03	0.03	0.27	0.34	0.32
360	0.05	0.05	0.05	0.23	0.21	0.21
530	0.07	0.07	0.07	0.18	0.27	0.21
710	0.08	0.08	0.08	0.18	0.36	0.21
890	0.09	0.09	0.09	0.13	0.08	0.18
1800	0.10	0.10	0.11	0.10	0.08	0.12

Appendix C: Carbon fixation rates (ug/cm²/h) of periphyton samples (3 replicates) collected from Pine and Golden Ponds (14 October, 1985) in response to glyphosate (Roundup) additions.

Glyphosate (mg/L)	Pine			Golden		
	1	2	3	1	2	3
0	1.06	1.02	1.28	0.27	0.46	0.61
0.89	1.21	1.03	1.18	0.43	0.57	0.13
8.9	0.85	0.95	0.74	0.53	0.70	0.79
18	0.72	0.69	0.88	0.47	0.32	0.65
36	0.44	0.48	1.16	0.12	0.25	0.44
53	0.50	0.61	0.57	0.55	0.45	0.04
71	0.47	0.58	0.58	0.54	0.18	0.18
89	0.51	0.76	0.45	0.07	0.10	0.15
890	0.35	0.44	0.24	0.17	0.10	0.04
1800	0.28	0.31	0.22	0.10	0.13	0.12

Appendix D: Carbon fixation rates (ug/cm²/h) of periphyton samples (3 replicates) collected from Hike and Birch Ponds (17 October, 1985) in response to glyphosate (Roundup) additions.

Glyphosate (mg/L)	Hike			Birch		
	1	2	3	1	2	3
0	0.91	0.51	0.61	0.62	0.57	0.90
0.89	0.55	0.71	0.60	0.47	0.75	0.66
8.9	0.56	0.57	0.51	0.51	0.58	0.67
18	0.43	0.38	0.40	0.28	0.39	0.82
36	0.35	0.26	0.58	0.17	0.25	0.45
53	0.28	0.28	0.21	0.24	0.31	0.22
71	0.32	0.26	0.26	0.17	0.23	0.21
89	0.32	0.21	0.19	0.25	0.23	0.35
890	0.20	0.19	0.17	0.09	0.09	0.09
1800	0.21	0.14	0.16	0.08	0.09	0.06